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Synthesis of novel G factor- or chloroquine-artemisinin hybrids and conjugates with potent antiplasmodial activity

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ABSTRACT

A series of novel hybrids of artemisinin (ART) with either a phytormone endoperoxide G factor analogue (GMeP) or chloroquine (CQ) and conjugates of the same compounds with the polyamines (PAs) spermidine (Spd) and homospermidine (Hsd) were synthesized and their antimalarial activity were evaluated using the CQ-resistant *P. falciparum* FcB1/Colombia strain. The ART-GMeP hybrid **5** and compounds **9** and **10** which are conjugates of Spd and Hsd with two molecules of ART and one molecule of GMeP, were the most potent with IC_{50} values of 2.6, 8.4 and 10.6 nM, respectively. The same compounds also presented the highest selectivity indexes against the primary human fibroblast cell line AB943 ranging from 16,372 for the hybrid **5** to 983 for the conjugate **10** of Hsd.

Keywords: artemisinin, phytormones G-factors, chloroquine, hybrids, conjugates, antimalarials

Malaria is a serious parasitic disease affecting every year approximately 200 million of humans and causing almost half a million of deaths among infected individuals, according to WHO (World Health Organization). Two main families of drugs are essentially being used for the treatment of malaria. The first one is based on the alkaloid quinine (QN), its more potent analogue chloroquine (CQ) and other quinoline-based antimalarials (e.g. mefloquine or piperaquine), that have been developed thereafter (Figure 1).[1,2] Unfortunately, *P. falciparum* has already developed resistance for CQ and quinoline-based antimalarials. The second family

is based on the natural product Artemisin (ART), that was first isolated from the Chinese medicinal plant *Artemisia annua L.* in 1972 by Tu Youyou.[3] ART is a sesquiterpene lactone which incorporates an endoperoxide functionality demonstrated to be important for its antimalarial activity.[4] Because of its low bioavailability and an unfavorable pharmacokinetic profile *in vivo*, intensive research efforts have been concentrated to the development of improved hemisynthetic and fully synthetic ART analogs and derivatives. These efforts have been culminated to the development of the C-10 *oxa*-substituted ART-based drugs, such as the dihydroartemisinin (DHA), the lipophilic artemether, the hydrophilic sodium artesunate (Figure 1) but more importantly to the corresponding C-10-*carba*-substituted ART with improved ADMET properties. In that respect we can mention as attractive intermediate the acid (**1**)[8,9] or ART hybrids (see below), such as the anilide **2**.[9] In addition, C-10-*carba*-substituted ART dimers or trimers, molecules with two or even three ART moieties connected through symmetric or asymmetric linkers have shown more potent biological (antimalarial or anticancer) activity, depending from the nature of the linker.[10, 11,12] Unfortunately, *P. falciparum* resistance to ART derivatives in the South East Asia and particularly in Cambodia has been observed. [13] In a seminal work,[5] molecular marker of ART resistance that is the kelch propeller protein (K13-propeller) has recently been identified and should be important in the continuing efforts to restrain ART resistance.[6,7]

Inspired by ART, many groups developed methods for constructing trioxanes, entities known to exhibit antimalarial activity in a similar manner to ART. Synthetic molecules of this type with strong activities have been reported by the groups of Posner, Kepler, Singh, Griesbeck.[14, 15] Of high importance are also the efforts of Meunier's group towards the development of trioxaquines that are molecules based on the «covalent bitherapy», obtained by covalent attachement of a trioxane entity to an aminoquinoline entity.[17, 18]

Since many years now, we have been interested in the naturally occurring phytormones known as G- factors (**G1**, **G2**, **G3**) synthesized from syncarpic acid and incorporating an endocyclic endoperoxide frame.[19] The natural compounds and many synthetic analogs have been synthesized.[20, 21] Among them, the methylated compound G3Me (Figure 1) and its derivatives have been shown to be potent antimalarial agents. In particular, the G3Me-derived amino-endoperoxide spiro compound (endoperoxide GMeP, **3**), suitable for the synthesis of G factor hybrids through a succinic acid type linker (compound **4**), has been developed and two compounds incorporating also CQ proved to be very active.[21]

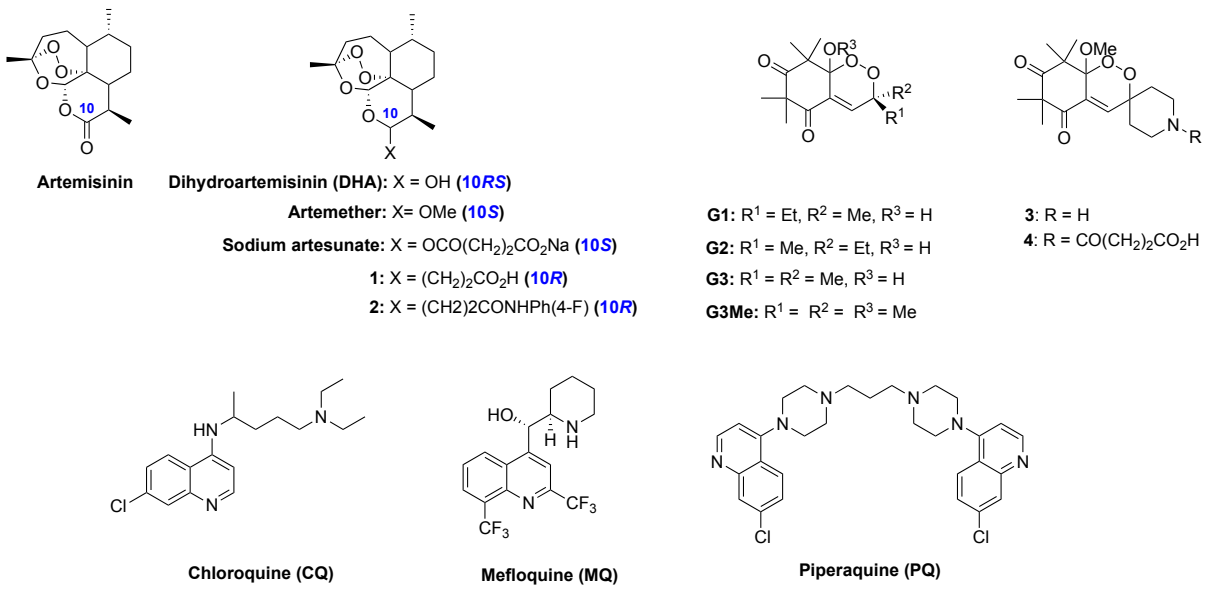


Figure 1. Molecular structures of known antimalarial agents.

Due to the emerging resistance of *P. falciparum* to artemisinins, WHO urgently recommends the use of Artemisinin-based Combination Therapies (ACTs), which involve the combination of a potent but short-lived ART derivative, e.g. artemether or sodium artesunate, with one or even two longer-lasting partner drugs with a different mechanism of action, e.g. a quinoline-based drug. Such combinations are for example "artesunate-MQ" or "artesunate-MQ-PQ". Recently, the combination of two antimalarial drugs in one single molecular entity (coined as hybrid or conjugate) has been described as being more efficient against diseases, such as malaria and cancer. Hybrid molecules have probably less chance to develop drug resistance.[1] Examples of ART–CQ (**S1**-**S2**)[1,11] or a G-factor–CQ (**S3**) hybrids,[12] and of conjugates (**S4**-**S7**) of ART with the naturally occurring polyamines (PAs), e.g. spermidine (Spd) or spermine (Spm),[22- 24] of relevance to the present work are provided in Figure S1 (Supporting Information). It should be noted that naturally occurring and synthetic PAs, their analogs and conjugates with other biologically active molecules are compounds with a variety of interesting biological properties.[25, 26] Conjugation of organic or bioorganic molecules with PAs has been used as a mean to improve or combine the biological properties of the constituent molecules. PAs can form the basis for the development of novel pharmaceuticals.[27, 28]

Accordingly, we now wish to report our first results on the synthesis of two types of artemisinin hybrids/conjugates with the endoperoxide GMeP (**3**) or the 4-aminosubstituted 7-chloroquinoline (ACQ) core of CQ, and the biological evaluation of their antimalarial potential. Hybrids of the first type are the compounds **5**, **6** and **7** (Figure 2) in which the two

pharmacophores are joined with an amide bond using suitable linkers. For the sake of comparison, the hybrid **8**[19] of endoperoxide-GMeP (**3**) with ACQ was also synthesized. Conjugates of the second type (Figure 2) are characterized by two molecules of ART and one molecule of endoperoxide-GMeP (compounds **9** and **10**) or ACQ (compounds **11** and **12**). For the sake of comparison, conjugates bearing two molecules of **3** and one molecule of ACQ (**13** and **14**) per molecule of PA were also synthesized.

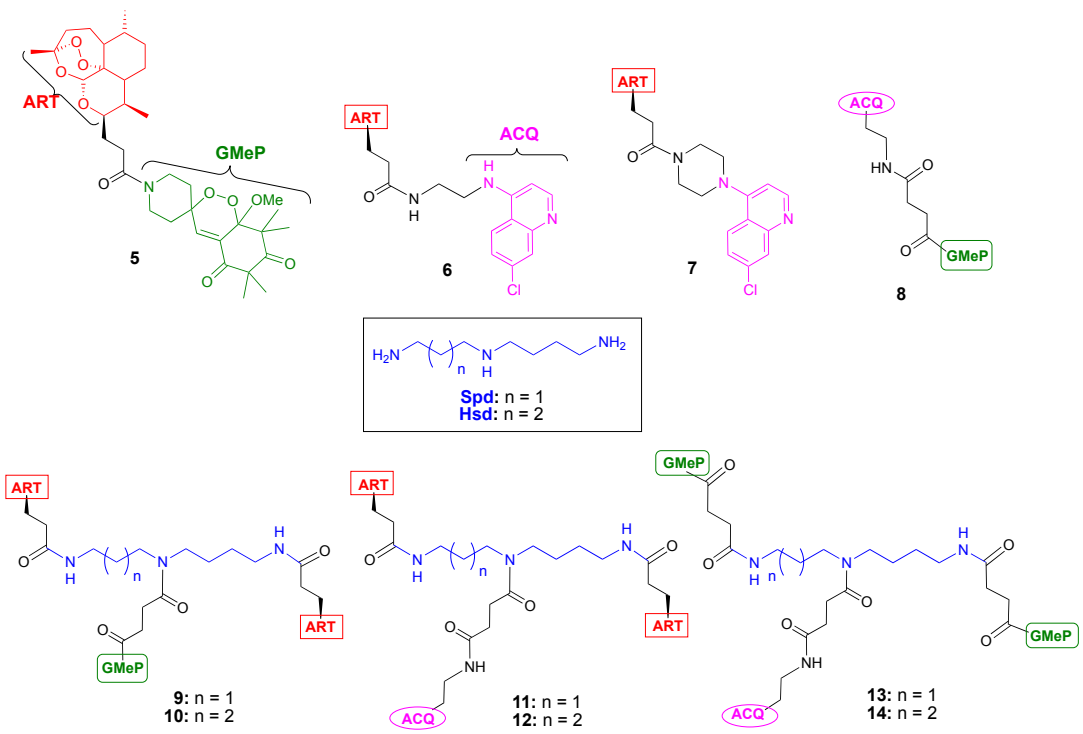


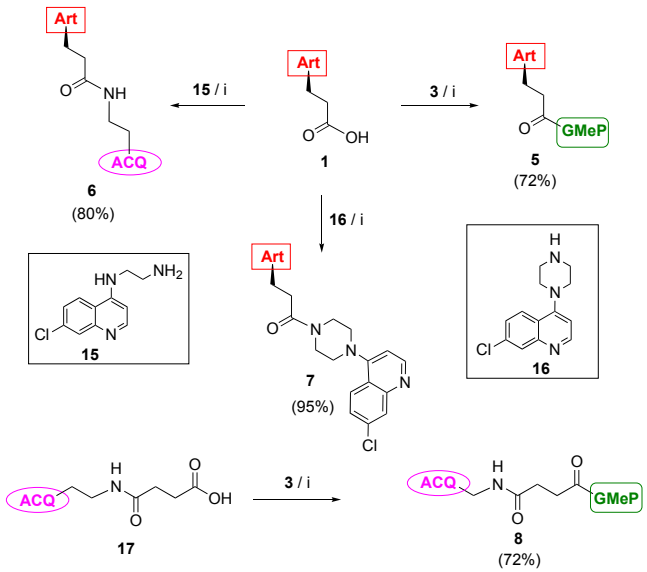
Figure 2. Structures of synthesized ART–endoperoxide GMeP (**5**), ART–ACQ (**6** and **7**) and ACQ–endoperoxide GMeP (**8**) hybrids, and ART–Spd and –Hsd mixed conjugates with endoperoxide GMeP and ACQ (**9**, **10** and **11**, **12**, respectively) and endoperoxide GMeP–Spd and –Hsd mixed conjugates with ACQ (**13** and **14**).

With these new hybrids/conjugates, we wished to determine the various factors affecting their potential antimalarial activity, such as (a) the influence of the other antimalarial agents linked with ART (comparing for example conjugates **5** and **6** or **9** and **11**), (b) the structure (conformationally restricted or not) or the length (Spd against Hsd chain) of the linker (comparing for example conjugates **6** and **7** or **9** and **10**, respectively) and (c) the number (one against two) of ART or endoperoxide-GMeP residues in the conjugate (comparing for example conjugates **5** and **9** or **8** and **13**, respectively).

The synthesis of ART conjugates **5**, **6** and **7** requires the ART-derived carboxylic acid **1,** as key-intermediate. This compound was prepared (Scheme S1, Supporting Information) in 58% overall yield from ART, according to a five-steps published procedure.[8,9] On the other hand, endoperoxide GMeP (**3**) was synthesized from syncarpic acid in a sequence of three *in situ* reactions (Mannich, dienol formation, oxygen uptake) followed by *tert*-butyloxycarbonyl (Boc) deprotection in an overall yield of 50%, according to our published procedure (Supporting Information).[21] The required syncarpic acid was synthesized (Supporting Information), essentially through a reported procedure starting from 2',4',6'-trihydroxyacetophenone.[29] An alternative synthesis of this key-intermediate has been described.[19]

Finally, the N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**15**) and the 7-chloro-4-(piperazin-1-yl)quinoline (**16**) were readily obtained in 56% and 76% yield, respectively, from the commercially available 4,7-dichloroquinoline through the nucleophilic aromatic substitution of the latter with 1,2-diaminoethane and piperazine, respectively (Scheme S1, Supporting Information).[21]

Having in our hands all the above needed intermediates we proceeded to the synthesis of the hybrids and conjugates as described in Schemes 1 and 2, respectively. Thus, condensation of acid **1** with amines **3** (GMeP), **15** and **16**, in the presence of the coupling agent HBTU and DIPEA, produced unexceptionally the anticipated conjugates **5**, **6** and **7** in 95%, 80% and 72% yields, respectively (Scheme 1).



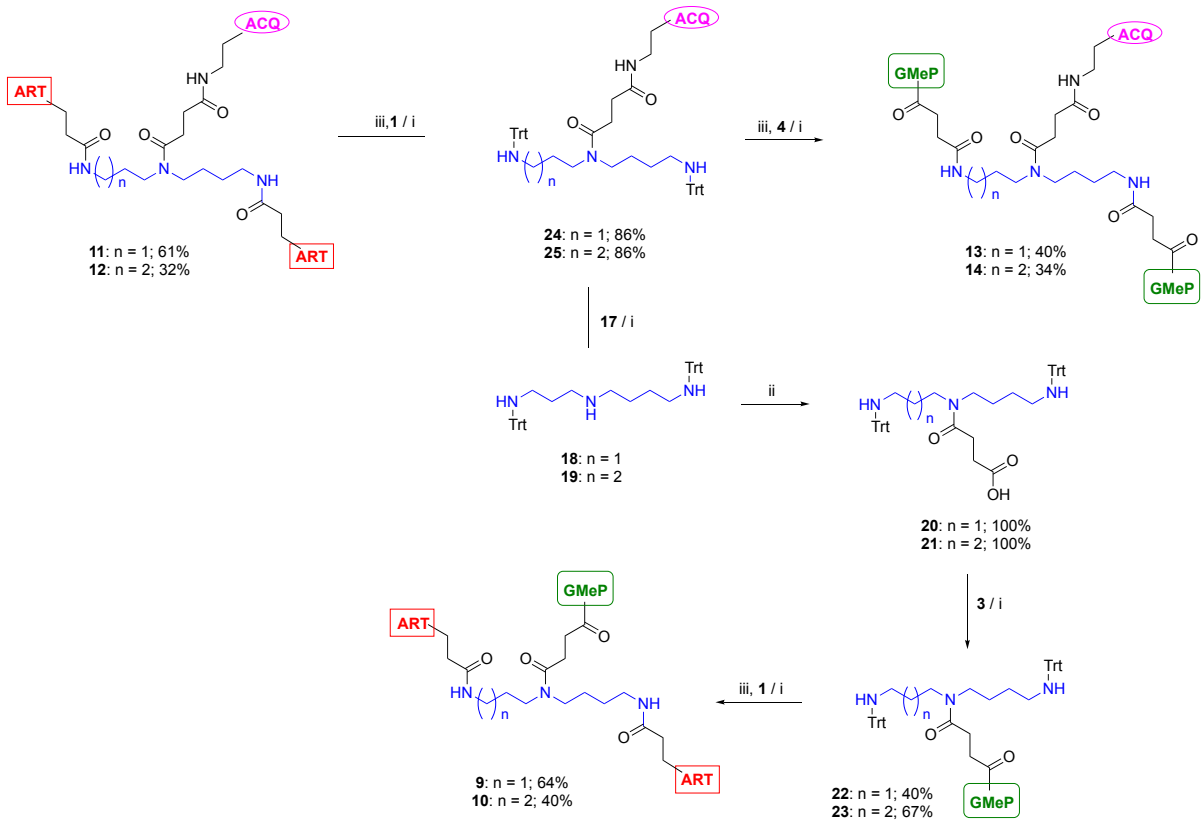
Scheme 1. Synthesis of ART–endoperoxide GMeP (**5**), ART–ACQ (**6** and **7**) and ACQ–endoperoxide GMeP (**8**) hybrids. *Reagents and reaction conditions*: (i) HBTU, DIPEA, CH_2Cl_2, 0 °C to rt, 3-12 h.

On the other hand, the synthesis of the conjugate **8** involved the initial reaction of amine **15** with succinic anhydride to give the corresponding carboxylic acid **17** in 100% yield. This acid was then coupled with endoperoxide GMeP (**3**), also in the presence of HBTU and DIPEA, to give conjugate **8** in 72% yield.

The synthesis of ART-Spd and Art-Hsd mixed conjugates **9**, **10**, **11** and **12** with endoperoxide GMeP **(3)** or ACQ, as well as the endoperoxide GMeP-Spd and endoperoxide GMeP-Hsd mixed conjugate **13** and **14** with ACQ, required the availability of suitably protected triamines, such as the N^1,N^8-ditritylspermidine (**18**)[30, 31] and N^1,N^9-ditritylhomospermidine (**19**). These compounds are readily available, in 48% overall yield, from butane-1,4-diamine (putrescine, Put) through a three-steps sequence, depicted in Scheme S1 (Supporting Information).

Reaction of PA derivatives **18** and **19** with succinic anhydride gave the anticipated monocarboxylic acid derivatives **20** and **21**, respectively, in quantitative yield. These compounds were then coupled with endoperoxide GMeP (**3**) in the presence of HBTU and DIPEA to give the protected PA conjugates **22** and **23** in 40% and 67% yield, respectively. The final products **9** and **10** were obtained in 64% and 40% yield, respectively, upon *N*-deprotection followed by coupling with ART-derived acid **1**, in the presence of HBTU and DIPEA (Scheme 2).

On the other hand, coupling of partially protected PAs **18** and **19** with the ACQ-derived acid **17**, in the presence of HBTU and DIPEA, produced PA conjugates **24** and **25** in 86% yield. From these conjugates, the final products **11** and **12** were unexceptionally obtained in 61% and 32% yields upon detritylation and coupling with ART-derived acid **1**. Similarly, the conjugates **13** and **14** were readily obtained (Scheme 2) from the same polyamine conjugates **24** and **25**, in 40% and 34% yield, respectively, upon deprotection of the latter followed by coupling with endoperoxide GMeP-derived acid **4**. The latter was readily obtained in 98% from the reaction of endoperoxide GMeP and succinic anhydride (Scheme S1). The experimental details for the synthesis of (a) all above described fragments, which are necessary for the assembly of the proposed hybrids/conjugates, and (b) the final products, that is hybrids/conjugates **5-14,** are provided in the Supporting Information section.



Scheme 2. Synthesis of ART–Spd and –Hsd mixed conjugates with endoperoxide GMeP or ACQ (**9-12**) and endoperoxide GMeP–Spd and –Hsd mixed conjugates with ACQ (**13** and **14**). *Reagents and reaction conditions*: (i) HBTU, DIPEA, CH_2Cl_2, 0 °C to rt, 3-12 h; (ii) succinic anhydride, DMAP, CH_2Cl_2, 0 °C to rt, 12 h; (iii) 3-6% TFA in CH_2Cl_2, CF_3CH_2OH, 0 °C, 2-12 h.

The synthesized conjugates were evaluated for their antiplasmodial activity against the CQ-resistant *P. falciparum* FcB1/Colombia strain, according to published procedure.[32,33] The cytotoxicity of the compounds was evaluated using the primary human fibroblast cell line AB943, also according to published procedure,[34] which allowed the calculation of their selectivity index (SI). In Table 1 (see also Table S1, Supporting Information), the measured IC_{50} values or the % inhibition at 10 nM, in cases IC_{50} values could not be determined, and SIs for the synthesized hybrids/conjugates are reported. IC_{50} values for ART, CQ and endoperoxide GMeP (**3**) are also provided for the sake of comparison.

Table 1. Evaluation of antiplasmodial activities of synthesized compounds with CQ-resistant *P. falciparum* FcB1/Colombia strain and selectivity indexes (SI).

Compd	IC_{50} (nM) or % inhibition at 10 nM	SI	Compd	IC_{50} (nM) or % inhibition at 10 nM	SI
5	2.6±1.0	16,372	**12**	52.3±15.9	328
6	11%	–	**13**	>167	–
7	11%	–	**14**	>167	–
8	124.3±40.4	144	**ART**	55±13.6	>4,545
9	8.4±3.7	2,108	**CQ**	72±7.4	347
10	10.6±2.3	983	**3**	>250	–
11	33%	–			

From Table 1 it is apparent that three out of seven of the synthesized hybrids/conjugates of ART, incorporating one or two molecules of ART per hybrid/conjugate, are significantly more potent (IC_{50} values ranging from 2.6 to 10.6 nM) than the parent compound, one conjugate (**12**) was almost equipotent to ART but with at least 13 times lower SI value and the rest showed low inhibition values (11-33%) at 10 nM. In particular the hybrid **5** of ART with endoperoxide GMeP is the most potent in this series of compounds with an IC_{50} value 21 times more potent than that of ART. Interestingly, this compound appears to be the safest compound for use as antimalarial (SI = 16,372) as its SI is significantly higher than that of ART and ca. 8-17 times higher than the other two best synthesized conjugates (**9** and **10**). A comparison of hybrid **5** (IC_{50}=2.6, SI=16,372), bearing one molecule of ART, with the conjugates **9** (IC_{50}=8.4, SI=2,108) and **10** (IC_{50}=10.6, SI=983), bearing two molecules of ART held together through a PA chain, showed that a second ART molecule does not offer any particular advantage but also leads to significant lowering of the SI value. On the other hand, comparison of the two conjugates **9** and **10**, differing only in the length of the PA chain, shows that the two compounds have comparable IC_{50} but the one with the Hsd longer chain is ca. 2.1 times less safe than the one with the Spd shorter chain. On the other hand, the hybrids **6** and **7** of ART with the CQ core show identically low inhibition values (11% inhibition. at 10 nM), indicating that (a) the CQ core does not offer any advantage to ART and (b) the structure of the diamine linker seems not to be important for the antiplasmodial activity.

Comparison of the couples of conjugates **9** (IC_{50}=8.4, SI=2,108) and **11** (33% inh. at 10 nM) and **10** (IC_{50}=10.6, SI=983) and **12** (IC_{50}=52.3, SI=460), bearing the Spd and the Hsd PA core linkers respectively, two ART molecules and either a molecule of the endoperoxide GMeP

or the CQ core, shows that the endoperoxide GMeP substructure is more important for biological activity than the CQ core providing safer conjugates. Finally, the hybrid **8** of endoperoxide GMeP with the CQ core is almost 2.5 times less active than ART having almost 10 times higher SI value. Moreover, comparison of conjugate **8** (IC_{50}=124.3, SI=144) with the conjugates **13** (IC_{50}>167) and **14** (IC_{50}>167), incorporating two molecules of endoperoxide GMeP per molecule of conjugate, shows that the former is slightly more active than the latter conjugates.

In conclusion, a series of novel hybrids of ART with either endoperoxide GMeP or the ACQ core of CQ and conjugates of the same compounds with the PAs Spd (an asymmetric PA) and Hsd (a symmetric, with longer chain, PA), bearing either two molecules of ART or of endoperoxide GMeP, were synthesized and their antiplasmodial activities and selectivity indexes (SI) were evaluated using the CQ-resistant *P.falciparum* FcB1/Colombia strain and the primary human fibroblasts cell line AB943 (for SI calculations), respectively. These studies showed that the most potent combination with the highest SI value was that between ART and the endoperoxide GMeP, in particular when the two molecules participate in the construct (hybrid/conjugate) in the ratio 1:1, that is without the intervention of a PA chain. Combinations between endoperoxide GMeP and ACQ seem not to be particularly effective, whereas the length of the PA chain or the geometry (symmetric or asymmetric) of the construct, defined by the PA used, does not seem to play any significant role. It seems that combinations of two endoperoxide-type compounds can form the basis for the development of more potent and safer antimalarial and therefore we are currently engaged in this line of research. Moreover, it should be important to study in a further work, if the three hybrid compounds possessing two different trioxane frames and different linkers between them can affect the spread of ART resistance and mitigate its impact on malaria treatment.

Supporting Information. Synthetic and Bioassay protocols, as well as, copies of ^{1}H and ^{13}C NMR spectra for all the synthesized compounds.

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Dedication: This article is dedicated to the memory of our good friend, Professor Maurizio Botta.

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Author Contributions: D. Pepe and D. Toumpa carried out the synthetic work and prepared the Supporting Information; C.M. Athanassopoulos conceived and directed the whole project and wrote part of the manuscript; M. Baltas co-directed the project, followed up the program in Toulouse and wrote part of the manuscript; C.A.B. followed up synthetic steps in Toulouse of the G3 endoperoxide part and a final compound; C.M. supported analytical and preparative chromatography; P. Grellier and E. Mouray directed and performed all biological studies; D. Papaioannou supervised the polyamine synthetic part and wrote part of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations: ACQ, 4-amino-7-chloroquinoline; ACTs, Artemisinin-based combination therapies; ADMET, Absorption, Distribution, Metabolism, Excretion and Toxicity; ART, artemisinin; Boc, *tert*-butoxycarbonyl; CQ, chloroquine; DIPEA, diisopropylethylamine; DMAP, 4-Dimethylaminopyridine; GMeP, phytormone endoperoxide G-factor analogue; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Hsd, homospermidine; MQ; mefloquine; PAs, polyamines; PQ, piperaquine; Put, putrescine; QN, quinine; rt, room temperature; SI, selectivity index; Spd, spermidine; Spm, spermine; TFA, Trifluoroacetic acid; WHO, World Health Organization.

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Synthesis of novel G factor- or chloroquine-artemisinin hybrids and conjugates with potent antiplasmodial activity

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